

WHAT IS CLAIMED IS:

1. A method of producing a glycoprotein with reduced complex carbohydrates comprising:
- 5 a. introducing and expressing a polynucleotide encoding a glycoprotein into a mammalian cell;
- b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
- c. isolating the lectin resistant mammalian cell;
- 10 d. culturing said lectin resistant mammalian cell, expressing said glycoprotein; and
- e. collecting the glycoprotein from said lectin resistant cells.
2. The method of Claim 1, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and
- 15 wheat germ agglutinin.
3. The method of Claim 2, wherein said lectin is ricin.
4. The method of Claim 1, wherein said glycoprotein is a lysosomal hydrolase.
5. The method of Claim 4, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -L-iduronidase, α -galactosidase A,
- 20 arylsulfatase, N-acetylgalactosamine-6-sulfatase or β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C,
- 25 Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1}

Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

- 5 6. The method of Claim 5, wherein said lysosomal hydrolase is acid α -glucosidase.
7. The method of Claim 1, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.
8. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises
10 SEQ ID NO:2.
9. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.
10. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises SEQ ID NOS:4, 5 and 7.
11. The method of Claim 7, wherein the GlcNAc-phosphotransferase is encoded
15 by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:1.
12. The method of Claim 7, wherein the GlcNAc-phosphotransferase comprises an α -subunit and a β subunit, which are encoded by a nucleotide sequence
20 comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:3; and a γ subunit, which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a

nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:6.

13. The method of Claim 7, further comprising purifying said glycoprotein after said contacting.

5 14. The method of Claim 7, wherein after said contacting with GlcNAc-phosphotransferase the method further comprises contacting with said glycoprotein with a phosphodiester α -GlcNAcase.

15. The method of Claim 14, wherein said phosphodiester α -GlcNAcase comprises an amino acid sequence of SEQ ID NO:18.

10 16. The method of Claim 14, wherein said phosphodiester α -GlcNAcase is encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide sequence that hybridizes under stringent conditions to the complement of SEQ ID NO:17.

15 17. The method of Claim 14, further comprising purifying said glycoprotein after said contacting.

18. A glycoprotein produced by the method of Claim 1.

19. A method of producing a glycoprotein deficient in complex carbohydrates comprising:

- 20
- a. introducing and expressing a polynucleotide encoding a glycoprotein into a mammalian cell;
 - b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
 - c. isolating the lectin resistant mammalian cell;
 - d. culturing said lectin resistant mammalian cell; and
 - 25 e. collecting the glycoprotein from said lectin resistant cells.

20. The method of Claim 19, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

21. The method of Claim 20, wherein said lectin is ricin.

5 22. The method of Claim 19, wherein said glycoprotein is a lysosomal hydrolase.

23. The method of Claim 22, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -L-iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

24. The method of Claim 23, wherein said lysosomal hydrolase is acid α -glucosidase.

20 25. The method of Claim 19, further comprising contacting the collected glycoprotein with a GlcNAc-phosphotransferase.

26. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2.

27. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises
SEQ ID NO:2 and SEQ ID NO:7.

28. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises
SEQ ID NOS:4, 5 and 7.

5 29. The method of Claim 25, wherein the GlcNAc-phosphotransferase is encoded
by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence
that hybridizes under stringent conditions to the complement of SEQ ID NO:1.

10 30. The method of Claim 25, wherein the GlcNAc-phosphotransferase comprises
an α -subunit and a β subunit, which are encoded by a nucleotide sequence
comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under
stringent conditions to the complement of SEQ ID NO:3; and a γ subunit,
which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a
nucleotide sequence that hybridizes under stringent conditions to the
complement of SEQ ID NO:6.

15 31. The method of Claim 25, further comprising purifying said glycoprotein after
said contacting.

32. The method of Claim 25, wherein after said contacting with GlcNAc-
phosphotransferase the method further comprises contacting with said
glycoprotein with a phosphodiester α -GlcNAcase.

20 33. The method of Claim 32, wherein said phosphodiester α -GlcNAcase
comprises an amino acid sequence of SEQ ID NO:18.

34. The method of Claim 32, wherein said phosphodiester α -GlcNAcase is
encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide

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sequence that hybridizes under stringent conditions to the complement of SEQ
ID NO:17.

35. The method of Claim 32, further comprising purifying said glycoprotein after
said contacting.

5 36. A glycoprotein produced by the method of Claim 19.

37. A method of making a mammalian cell that produces glycoproteins having
reduced complex carbohydrates comprising

a. introducing and expressing a polynucleotide encoding a glycoprotein
into a mammalian cell;

10 b. culturing the mammalian cell in the presence of a lectin in an amount
sufficient to obtain a lectin resistant mammalian cell;

c. isolating the lectin resistant mammalian cell;

15 38. The method of Claim 37, wherein said lectin is selected from the group
consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and
wheat germ agglutinin.

39. The method of Claim 38, wherein said lectin is ricin.

40. The method of Claim 38, wherein said glycoprotein is a lysosomal hydrolase.

41. The method of Claim 40, wherein said lysosomal hydrolase is selected from
the group consisting of α -glucosidase, α -L-iduronidase, α -galactosidase A,
20 arylsulfatase, N-acetylgalactosamine-6-sulfatase or β -galactosidase,
iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase,
Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -
glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase,
Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C,
25 Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1}

Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

- 5 42. The method of Claim 41, wherein said lysosomal hydrolase is acid α -glucosidase.
43. A mammalian cell that produces glycoproteins having reduced complex carbohydrates obtained by the method of Claim 37.
- 10 44. A method of treating a patient suffering from a lysosomal storage disease comprising administering to said patient a lysosomal hydrolase in an amount sufficient to treat said disease, wherein said lysosomal hydrolase is obtained by a method comprising:
- 15 a. introducing and expressing a polynucleotide encoding said lysosomal hydrolase into a mammalian cell;
- b. culturing the mammalian cell in the presence of a lectin in an amount sufficient to obtain a lectin resistant mammalian cell;
- c. isolating the lectin resistant mammalian cell;
- d. culturing said lectin resistant mammalian cell;
- e. collecting the lysosomal hydrolase from said lectin resistant cells;
- 20 f. contacting the collected lysosomal hydrolase with a GlcNAc-phosphotransferase; and
- g. contacting said lysosomal hydrolase with a phosphodiester α GlcNACase after said contacting with a GlcNAc-phosphotransferase.

45. The method of Claim 44, wherein said lectin is selected from the group consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

46. The method of Claim 45, wherein said lectin is ricin.

5 47. The method of Claim 45, wherein said lysosomal hydrolase is selected from the group consisting of α -glucosidase, α -L-iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, 10 Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, 15 Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase and Sphingomyelinase.

48. The method of Claim 47, wherein said lysosomal hydrolase is acid α -glucosidase.

49. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises 20 SEQ ID NO:2.

50. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises SEQ ID NO:2 and SEQ ID NO:7.

51. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises
SEQ ID NOS:4, 5 and 7.

52. The method of Claim 44, wherein the GlcNAc-phosphotransferase is encoded
by a nucleotide sequence comprising SEQ ID NO:1 or a nucleotide sequence
5 that hybridizes under stringent conditions to the complement of SEQ ID NO:1.

53. The method of Claim 44, wherein the GlcNAc-phosphotransferase comprises
an α -subunit and a β subunit, which are encoded by a nucleotide sequence
comprising SEQ ID NO:3 or a nucleotide sequence that hybridizes under
stringent conditions to the complement of SEQ ID NO:3; and a γ subunit,
10 which is encoded by a nucleotide sequence comprising SEQ ID NO:6 or a
nucleotide sequence that hybridizes under stringent conditions to the
complement of SEQ ID NO:6.

54. The method of Claim 44, wherein said phosphodiester α -GlcNAcase
comprises an amino acid sequence of SEQ ID NO:18.

15 55. The method of Claim 44, wherein said phosphodiester α -GlcNAcase is
encoded by a nucleotide sequence comprising SEQ ID NO:17 or a nucleotide
sequence that hybridizes under stringent conditions to the complement of SEQ
ID NO:17.

56. A method of producing a glycoprotein with reduced complex carbohydrates
20 comprising:

- a. a step for introducing and expressing a polynucleotide encoding a
glycoprotein into a mammalian cell;
- b. a step for selecting a mammalian cell expressing said glycoprotein that
is resistant to a lectin;

- c. a step for culturing said lectin resistant mammalian cell, expressing said glycoprotein; and
- d. a step for collecting the glycoprotein from said lectin resistant cells.

57. The method of Claim 56, wherein said lectin is selected from the group

5 consisting of ricin, concanavalin A, erthroglutinin, lymphoagglutinin, and wheat germ agglutinin.

58. The method of Claim 57, wherein said lectin is ricin.

59. The method of Claim 56, wherein said glycoprotein is a lysosomal hydrolase.

60. The method of Claim 59 wherein said lysosomal hydrolase is selected from

10 the group consisting of α -glucosidase, α -L-iduronidase, α -galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase or β -galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, β -glucuronidase, Heparan N-sulfatase, N-Acetyl- α -glucosaminidase, Acetyl CoA- α -glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, 15 Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid β -galactosidase G_{M1} Galglioside, Acid β -galactosidase, Hexosaminidase A, Hexosaminidase B, α -fucosidase, α -N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal 20 Sphingomyelinase and Sphingomyelinase.

61. The method of Claim 60, wherein said lysosomal hydrolase is acid α -glucosidase.

62. The method of Claim 56, further comprising a step for transferring a N-acetylglucosamine-1-phosphate from UDP-GlcNAc to said glycoprotein.

63. The method of Claim 62, further comprising a step for purifying said glycoprotein comprising a N-acetylglucosamine-1-phosphate.

64. The method of Claim 62, further comprising a step for removing an N-acetylglucosamine from said glycoprotein.

5 65. A glycoprotein produced by the method of Claim 56.

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